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Source characterization and seasonal variations of atmospheric polycyclic aromatic hydrocarbons at an industrial and semi-urban area through a local-scale biomonitoring network using *T. capillaris*

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ABSTRACT

Designing a network of instrumental monitoring to obtain polycyclic aromatic hydrocarbon (PAH) concentration data at many sampling sites simultaneously is difficult due to the high cost of equipment and the problem of identifying suitable sites for installation. The use of biomonitors allows accurate approximations of air pollution, covering different working scales and levels of complexity, based on the number of individuals collected or transplanted. A monitoring network, consisting of biomonitors of *Tillandsia capillaris* transplanted for four seasons, was designed in order to assess the effects of different emission sources and the atmospheric dispersion of PAHs at a local scale, in the town of Malagueño, Argentina. Out of the sixteen priority control PAHs listed by the United States Environmental Protection Agency (USEPA) for their mutagenic and carcinogenic properties, thirteen of them were analyzed in this study. In addition, several physiological parameters were quantified in order to try to relate the damage in the transplanted biomonitors with the air quality in the sector. The biomonitoring study allowed the spatial (in 300 km^2) and temporal (over one year) variabilities in the concentrations of the PAHs emitted from multiple sources in a complex scenario to be assessed, thereby identifying marker elements from the emissions of a cement plant, biomass burning and traffic. The present study represents a different approach for analyzing the behavior of sources of emission of PAHs in space and time, and the results obtained can be compared with related sources from other regions of the world.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) comprise a large group of organic compounds containing two or more fused aromatic rings. PAHs are formed mainly as a result of pyrolytic processes, especially in the incomplete combustion of organic materials during anthropogenic activities such as the use of coal, crude oil and natural gas combustion, burning or incineration of waste, vehicular traffic, cooking and consumption of tobacco, as well as natural processes such as forest fires [1].

Due to their mobility, persistence and tendency to bioaccumulate and because of their toxic effects on human health, PAHs have been included in the Convention on the Transboundary Air Pollution Protocol on a large scale for Persistent Organic Pollutants [2,3] and the U.S. Environmental Protection Agency (USEPA) has incorporated 16 of them in the list of priority control pollutants, due to their mutagenic and carcinogenic properties [4].

As a result of their many sources and persistent characteristics, PAHs can disperse through atmospheric transport and exist almost everywhere [5]. In the atmosphere, they may be present in the gas phase, adsorbed on aerosols, or partitioned between the two phases, depending on temperature, vapor pressure, solubility of the compound, and the surface area and size of the particles in suspension [6]. Monitoring PAHs in air is usually performed by quantifying the PAHs in gaseous and particulate phases in the atmosphere, and while the PAHs in the gas phase are important, it is considered that those found in the particulate phase have a greater impact on human health [7].

It has been shown in spatial and temporal biomonitoring studies of these compounds [9–14] that plants can absorb and/or adsorb PAHs from the air [8]. Related to this, the Directive 2004/107/EC from the European Parliament and Council [15] has proposed the use of biomonitors as tools to evaluate the spatial deposition of PAHs. From exposure to PAHs resulting from the emission of anthropogenic activities, plants manifest effects with altered physiological and biochemical conditions [16], with these compounds inducing oxidative stress mediated by reactive oxygen species (ROS) [17]. The presence of PAHs in air can also cause chlorosis and deformation of trichomes with the consequent appearance of necrotic lesions in plants [18]. Therefore, a more accurate biomonitoring study should include the analysis of parameters that indicate physiological damage.

For PAH biomonitoring, the *Tillandsia* species (epiphytic plants, usually found in the Southern Hemisphere) have been shown to be suitable biomarkers. Of these, *Tillandsia usneoides* has been used as a bioindicator species of PAHs in Brazil [19], *Tillandsia caput-medusae*

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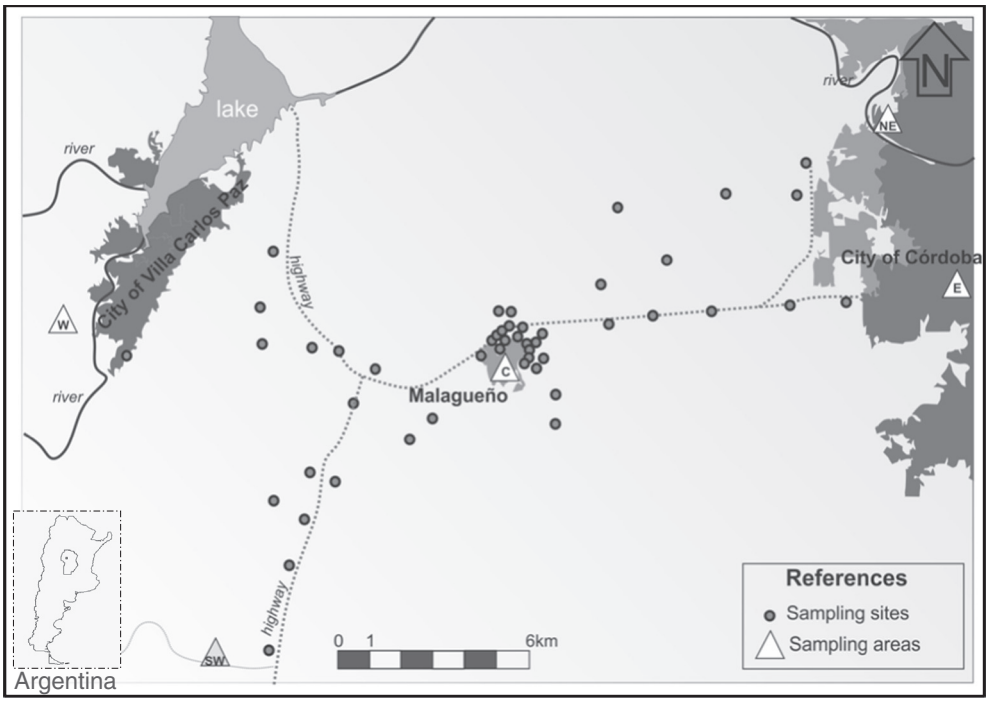

Fig. 1. Location of the study area and identification of the sampling subareas (NE, SW, E, W, C) in the town of Malagueño, province of Córdoba, Argentina.

and *Tillandsia bulbosa* have been used as bioindicators of PAHs in Italy [20], and *Tillandsia capillaris* has been used in a biomonitoring study in Germany [11] and in Argentina [14].

The present investigation was carried out in the town of Malagueño, in the province of Córdoba, Argentina, which has industries, biomass burning and vehicular traffic. All these sources involve combustion processes [21], with the probable emission of PAHs. The main objectives of the study were to: (1) analyze the spatial and temporal variability in the concentration of polycyclic aromatic hydrocarbons (PAHs) emitted on a local scale and accumulated by the biomonitoring species *T. capillaris* which was transplanted to the study area; (2) characterize the emission sources of PAHs and their effects on the air quality of the region; and (3) assess the physiological damage in *T. capillaris* transplants in relation to the accumulation of PAHs.

2. Materials and methods

2.1. Biomonitoring network

A monitoring network, consisting of transplanted biomonitors throughout 4 sampling periods, was designed in order to assess the effects of the different emission sources and their atmospheric

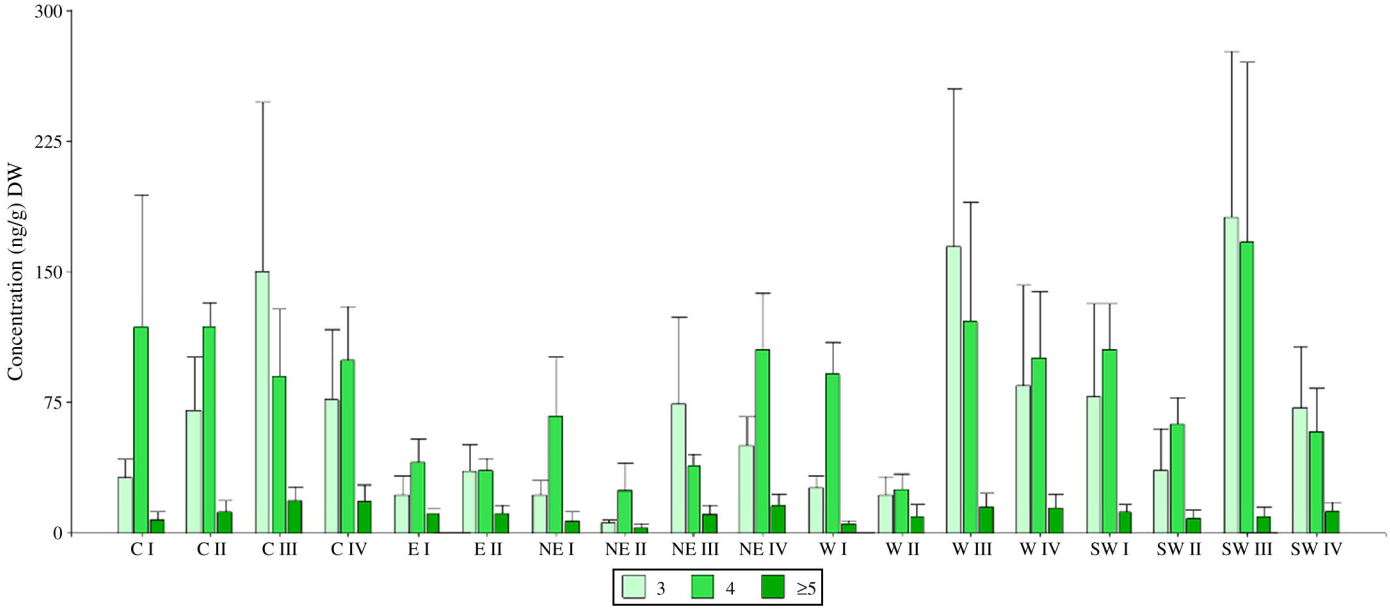

Fig. 2. Concentration values of polycyclic aromatic hydrocarbons ($ng \cdot g^{-1}$ DW) accumulated in *T. capillaris* exposed in the town of Malagueño, in relation to the number of aromatic rings and for each sub-sampling area in the four sampling periods: I (spring), II (summer), III (autumn), and IV (winter).

dispersions in the town of Malagueño, located 18 km SW of Córdoba city in Argentina (Fig. 1). In the study area, a cement plant was considered to be the main anthropogenic source of atmospheric pollutants, taking into account the results of previous studies undertaken in the area [11, 21–25]. The following subareas within the study area were selected, centering on the vicinities of the cement plant and covering a total area of approximately 300 km^2: C (Cement Plant); E (transect towards the east, in relation to "C"); NE (transect towards the northeast); SW (transect towards the southwest); W (transect towards the west). Detailed information regarding the study area and its subareas can be found in Section S1 (Supplementary material). The sampling sites were chosen with radial distributions surrounding the cement plant (C) and also with linear distributions in order to assess the effects of the prevalent winds from the northeast (NE) to the southwest (SW) as well as the influence of the study area on the cities of Cordoba and Villa Carlos Paz (E and W, respectively).

2.2. Biomonitors

Plants of *T. capillaris* Ruíz & Pav. f. *capillaris* were collected from standing trees at Dique la Quebrada, a natural reserve in the province of Córdoba located 38 km NW from the capital city. This area is considered to be unpolluted, where the baseline compositions of these plants have remained practically unchanged over the years. *T. capillaris* has been previously employed in other biomonitoring studies carried out by our research group and has been shown to act as a good biomonitor of response and accumulation in the assessment of atmospheric quality [21,23,25–28].

2.3. Active biomonitoring and exposure periods

Net bags, containing 8–10 plants, were prepared according to Wannaz and Pignata [29] and transplanted to the study area (n = 3 bags/site). These were placed 3 m above ground level and exposed for four periods of 3 consecutive months each, from September 2009 to September 2010 (Table S1 — Supplementary material). Once the exposure periods had been concluded, plants were collected and placed in paper bags. Part of the material was separated to determine the pH, EC (electric conductivity), water and sulfur content, whereas the rest was kept in plastic vials at −15 °C in the dark for subsequent physiological determinations and PAH accumulation. In order to establish the initial state of the samples before transplantation, basal samples from the original collection site were analyzed following the same procedures.

2.4. Extraction, cleanup and analysis of PAHs

2.4.1. PAH determination

PAH determination in leaves of *T. capillaris* was conducted using a methodology proposed by [30]. The extraction procedure was performed by placing 300 mg of the homogenized material with n-hexane and acetone (2 mL; 1:1, v/v) in the extraction cell, which was then immersed in a water bath (0 °C) and exposed to ultrasonic irradiation for 30 s using an ultrasonic probe with a 3 mm titanium microtip (BioLogics, Inc.). Then, the extracts were centrifuged for 15 min at 3000 rpm and the supernatant was filtered with a filter with pore size of 0.22 μm. Subsequently, the samples were allowed to dry in the dark at room temperature for 24 h, and after drying they were stored at −21 °C. Finally, samples were resuspended in 0.5 mL of acetonitrile (Baker) and analyzed by high performance liquid chromatography (HPLC Perkin Elmer Series 200) (Table S2). In the present study, a total of 13 PAHs from the 16 recommended by the USEPA for environmental monitoring were analyzed: acenaphthene (ACN), fluorene (FLN), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]

Table 1
Descriptive statistics of polycyclic aromatic hydrocarbons (ng·g^{-1} DW) quantified in *T. capillaris* for quarterly sampling periods I, II, III and IV in the town of Malagueño, province of Córdoba.

PAHs	Sampling period I: spring					Sampling period II: summer					Sampling period III: autumn					Sampling period IV: winter				
	n	Mean	S.E.	% C.V.	Min	Max	n	Mean	S.E.	% C.V.	Min	Max	n	Mean	S.E.	% C.V.	Min	Max	n	Mean	S.E.	% C.V.	Min	Max
ACN	30	22.32	2.69	64.02	1.87	61.81	50	20.14	2.28	79.89	1.53	55.35	42	111.8	12.32	71.44	12.18	321.8	22	31.30	4.09	61.25	8.20	69.32
FLN	30	86.13	20.71	131.7	8.74	477.6	50	71.43	10.07	99.64	1.30	300.6	42	67.93	18.12	172.8	2.24	557.4	22	53.60	3.64	31.86	18.85	78.25
PHE	30	31.89	7.13	122.4	1.12	111.5	50	91.23	14.88	115.3	0.46	409.9	42	417.8	47.63	73.89	0.60	1084	22	175.2	16.65	46.56	31.88	312.2
ANT	30	8.54	1.34	86.15	0.29	30.65	50	9.50	2.72	202.4	0.14	81.03	42	15.75	2.16	88.85	2.55	63.78	22	10.10	1.26	58.76	2.03	19.08
FLT	30	158.3	32.63	112.9	8.58	619.3	50	95.90	15.00	110.6	0.21	372.6	42	76.62	10.05	84.99	17.37	265.0	22	157.1	18.48	55.16	67.53	319.9
PYR	30	76.97	25.29	180.0	0.34	518.3	50	72.41	11.61	113.4	0.06	328.2	42	39.64	5.67	92.72	5.30	136.4	22	51.90	10.12	91.48	2.55	167.7
BaA	30	75.23	13.62	99.18	4.24	250.5	50	77.85	20.07	182.3	1.85	641.3	42	276.9	65.63	153.6	7.68	1690	22	181.3	18.47	47.77	21.76	298.0
CHR	30	38.16	10.17	146.0	0.37	216.0	50	58.90	17.83	214.0	0.21	617.8	42	44.23	6.72	98.49	1.46	164.2	22	33.24	6.73	95.03	4.71	104.8
BbF	30	23.13	3.02	71.49	5.08	60.47	50	33.92	3.64	75.90	2.16	95.29	42	42.00	3.78	58.32	4.48	89.16	22	43.96	3.00	31.96	24.77	73.67
BkF	30	4.13	0.76	100.2	0.36	16.42	50	4.35	0.47	75.78	0.55	12.31	42	3.95	0.38	61.93	0.47	12.20	22	4.77	0.37	36.28	2.74	7.70
BaP	30	3.46	0.41	64.30	0.90	9.64	50	2.93	0.32	76.80	0.18	8.08	42	4.15	0.66	103.7	0.19	22.48	22	3.82	0.29	35.24	1.78	6.29
DBA	30	4.54	0.88	105.7	0.84	18.40	50	6.35	1.43	158.9	0.19	44.84	42	14.27	2.23	101.4	0.87	53.70	22	9.46	0.89	44.14	2.80	15.13
BghiP	30	8.33	1.44	94.37	1.85	32.95	50	5.52	0.54	68.76	1.20	15.01	42	10.37	1.25	78.21	1.37	38.33	22	12.93	1.52	55.17	3.86	31.88
∑LMW		307.2			20.60	1301		288.2			3.64	1219		689.9			34.94	2292		427.3			128.5	798.7
∑HMW		234.0			13.98	1123		262.2			6.40	1763		435.5			21.82	2207		341.4			64.97	705.1
Total PAHs		541.2			34.58	2424		550.4			10.04	2982		1125			56.76	4499		768.8			193.5	1504

∑LMW: sum of low molecular weight PAHs; ∑HMW: sum of high molecular weight PAHs; S.E.: standard error; % C.V.: coefficient of variation percentage.

pyrene (BaP), dibenzo[a,h]anthracene (DBA) and benzo[g,h,i]perylene (BghiP). The results were expressed in $ng \cdot g^{-1}$ DW.

2.4.2. Quality control, reagents and cleaning procedures

For PAH determination, the solvents (hexane, acetone and acetonitrile) were of HPLC grade, and the water was purified by a Milli-Q system (Millipore Corp., Bedford, MA). All glassware and plastic materials were washed using a commercial detergent, then thoroughly rinsed with Milli-Q water and soaked in acetone for 24 h. The Certified Reference Material IAEA-140 OC (organochlorine compounds and petroleum hydrocarbons in seaweed), which contains the certified values for 10 PAHs (naphthalene, PHE, ANT, FLT, PYR, BaA, CHR, BkF, BaP and BghiP) was used to validate the method. Table S3 presents the values of certified material and those obtained experimentally in this work. The recovery percentages were found between 75% (ANT) and 125% (CHR), and naphthalene could not be measured since the emission peak overlapped with those belonging to the photosynthetic compounds. Polycyclic aromatic hydrocarbon standard solution (EPA 525 PAH Mix B) of 500 $mg \cdot mL^{-1}$ was purchased from Supelco (Argentina). Intermediate solutions were prepared by diluting the stock solution in acetonitrile and water (3:1 v/v), and these were used as daily calibrants (0.5, 5 and 10 $ng \cdot mL^{-1}$). This standard solution contained: FLN, acenaphthylene, PHE, ANT, PYR, BaA, CHR, BbF, BkF, BaP, DBA, indeno[1,2,3-cd]pyrene and BghiP. Acenaphthylene was not measured because it has no fluorescence, and indeno[1,2,3-cd]pyrene is eluted between DBA and BghiP. Laboratory blanks were prepared and measured, and these values were subtracted from the concentration values of PAHs in the samples analyzed (Table S4). From the assessment of the blanks during testing and analysis, all indications showed that this method was satisfactory.

2.5. Physiological determinations

The procedures followed for the quantification of pheophytin a (Pheo-a), pheophytin b (Pheo-b), malondialdehyde (MDA), hydroperoxy conjugated dienes (HPCDs), sulfur content (S) and a foliar damage index (FDI) have been previously described by Pignata et al. [26], and electric conductivity (EC) and simultaneous pH determinations were conducted using a methodology proposed by Pearson [31].

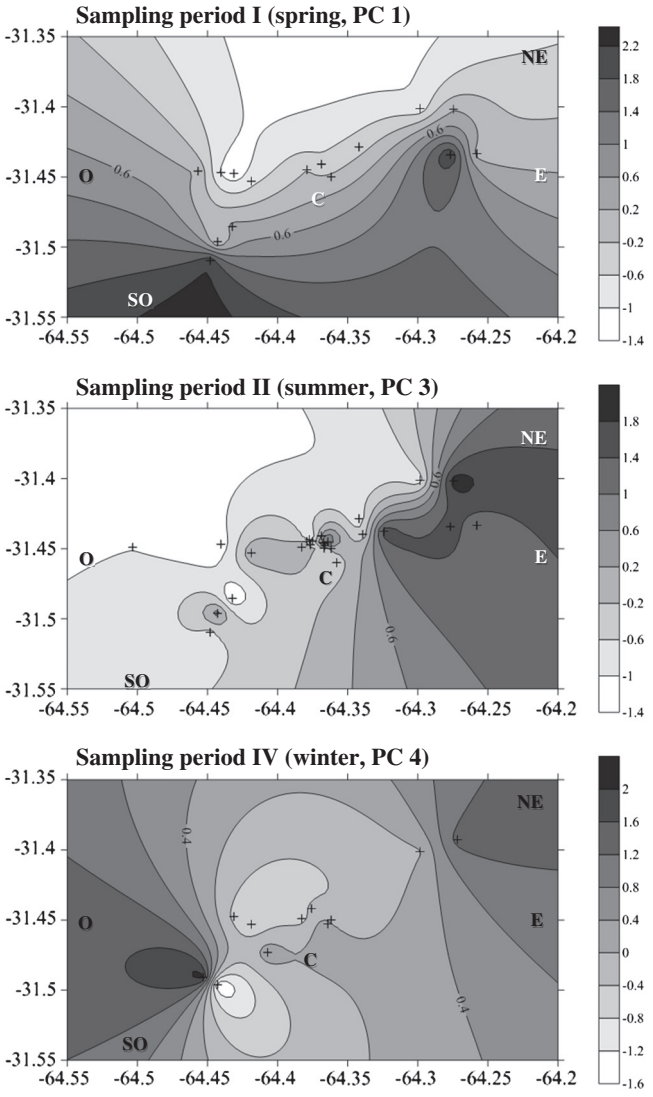

Fig. 3. Spatial distribution patterns of principal component values for the source of pollution identified: vehicular traffic.

Table 2
Factor loadings of polycyclic aromatic hydrocarbons accumulated in *T. capillaris* leaves at the sampling periods I, II, III and IV.

PAHs	Period I: spring				Period II: summer				Period III: autumn			Period IV: winter			
	PC 1	PC 2	PC 3	PC 4	PC 1	PC 2	PC 3	PC 4	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 4
ACN			0.509	**0.703**	0.350			**0.792**	**0.720**	0.341	0.193	0.240	**0.717**	0.227	0.305
FLN	0.400	−0.137	0.331	−0.566	**0.724**	0.477	0.109	0.155	**0.895**		−0.137	−0.267	−0.339	**−0.807**	−0.147
PHE	0.144	−0.163	0.205	**0.787**	0.175			**0.889**	0.578		0.327	0.390	−0.562	0.569	0.304
ANT	0.253	**0.924**	0.128	−0.160	**0.895**	0.161	0.231	0.149	0.181		**0.922**			0.605	
FLT		−0.285	**0.915**		0.277	**0.661**	0.381	0.277	**0.882**		0.327	0.208	**0.619**	0.603	−0.168
PYR	0.334	**0.889**	−0.183		**0.778**	−0.382		0.295	0.691	0.324	0.491		**0.924**		
BaA		0.122	**0.915**	0.210	**0.773**	0.443	0.246	0.230	**0.906**	−0.143	0.237	**0.954**			
CHR	0.256	**0.918**			**0.836**		0.103	0.403	**0.743**		0.446		0.327	**0.781**	−0.355
BbF	**0.754**	0.352	0.298			**0.936**			0.506	0.573	0.394	**0.698**	0.521	0.158	−0.180
BkF	**0.932**	0.104			0.189	0.451	**0.747**		**0.847**	0.263		**0.710**	0.380	0.167	
BaP	**0.773**	0.457	0.251	0.239	0.318	0.306	**0.648**	0.475	0.250	**0.673**	0.149	**0.900**			−0.153
DBA	**0.777**	0.455	−0.207	−0.189	**0.801**	0.331	0.416	0.130	−0.283	**0.866**			0.208	−0.350	**0.805**
BghiP	0.660	0.523	−0.211		0.107		**0.904**		0.131	**0.716**		−0.136		0.190	**0.867**
Eigen value	3.509	3.453	2.384	1.615	4.249	2.329	2.263	2.091	5.451	2.378	1.806	3.062	2.755	2.584	1.832
Variance %	26.995	26.560	18.335	12.482	32.684	17.915	17.411	16.081	41.932	18.293	13.893	23.554	21.190	19.877	14.089
Cumulative %	26.995	53.556	71.891	84.319	32.684	50.600	68.011	84.092	41.932	60.226	74.119	23.554	44.744	64.620	78.709

Values of dominant elements in each factor are reported in bold. Coefficient values with an absolute value <0.1 were suppressed.
Extraction method: PCA and rotation method: varimax with Kaiser Normalization.

2.6. Statistical analysis

Assumptions of normality were tested using the Shapiro–Wilk test, and non-normal distributed variables were transformed before carrying out parametric statistics. In order to analyze comparatively the accumulation of each PAH, the compounds accumulated in the biomonitors were assessed considering the content of these found in the basal samples (exposed-to-basal ratio, "*E–B ratio*") as described by Frati et al. [32] with the aim of analyzing the comparative accumulation. A one way analysis of variance (ANOVA) was performed for each PAH and physiological variable considering the different subareas (C, NE, E, W and SW) and sampling periods (I, II, III and IV). When the ANOVA null hypothesis was rejected (significance level <0.05), post-hoc comparisons were performed in order to investigate differences between pairs of means (Least Significant Difference, LSD). Furthermore, a Principal Component Analysis (PCA) was carried out, which is generally considered to be able to identify potential sources of air pollution in a study area, and the identified associations were mapped to represent the spatial distribution of each major component and associated pollutants to their potential sources of emission in the study area.

3. Results and discussion

3.1. PAHs accumulated in T. capillaris transplants

In order to establish a relationship between the PAHs, their volatility and seasonal variations in the different subareas, these compounds were grouped according to the number of aromatic rings present in their structures (Fig. 2) with PAHs being classified according to the USEPA and the Agency for Toxic Substances and Disease Registry (ATSDR) as follows: 3 aromatic rings: ACN, FLN, PHE and ANT; 4 aromatic rings: FLT, PYR, BaA and CHR, which can be grouped as compounds of low molecular weight (LMW PAHs); and greater than or equal to 5 aromatic rings: BbF, BkF, BaP, DBA and BghiP, under the category of compounds of high molecular weight (HMW PAHs) [33]. In Figs. S1 to S5 (Supplementary material) the concentration values of PAHs by compound for each subarea are presented. From Fig. 2 it can be seen that the major components were those of the 3 and 4 aromatic rings. In subareas W and SW, in autumn (sampling period III), a marked increase in the levels of PAHs could be observed (Figs. 2, S4 and S5), and this may have been due to major episodes of biomass burning, along with the most adverse weather conditions. In summer (sampling period II) at W (Figs. 2 and S4), the low concentration values of PAHs corresponded to the absence of fires at the waste dumping site during that season (as established by the firemen's hall data from the area). At the E subarea (Figs. 2 and S2) the concentration values were similar, indicating that the sources of atmospheric pollution by PAHs (predominantly vehicular traffic) emitted these pollutants steadily and apparently without any significant influence of the meteorological variables. This could also be observed for C, implying that the cement plant was also an active PAH emitter. In contrast, in the subareas W, NE and SW, the influence of seasonal variability on PAH concentrations (Fig. 2) was clearly observed. Here, it was also noticeable that within subareas there was a large variability in the concentration of PAHs (Fig. 2), which may have been caused by the distance between the sampling sites (approximately 300–500 m in radial distribution and 1–2 km in transects) and the local behavior of the heavier PAHs in relation to their emission sources.

The mean, minimum and maximum concentration values of PAHs ($ng \cdot g^{-1}$ DW) in *T. capillaris* transplanted to the study area are presented in Table 1, with the results of the analysis of variance (ANOVA) using "subareas" (C, SW, NE, E and W) and "sampling periods" (I, II, III and IV) as classification criteria being shown in Table S5. A comparison between the PAH concentrations obtained in this study and those found in previous studies in Cordoba and other regions of the world (using species from *Tillandsia* genus) is given in Table S6 and described in Section S2.

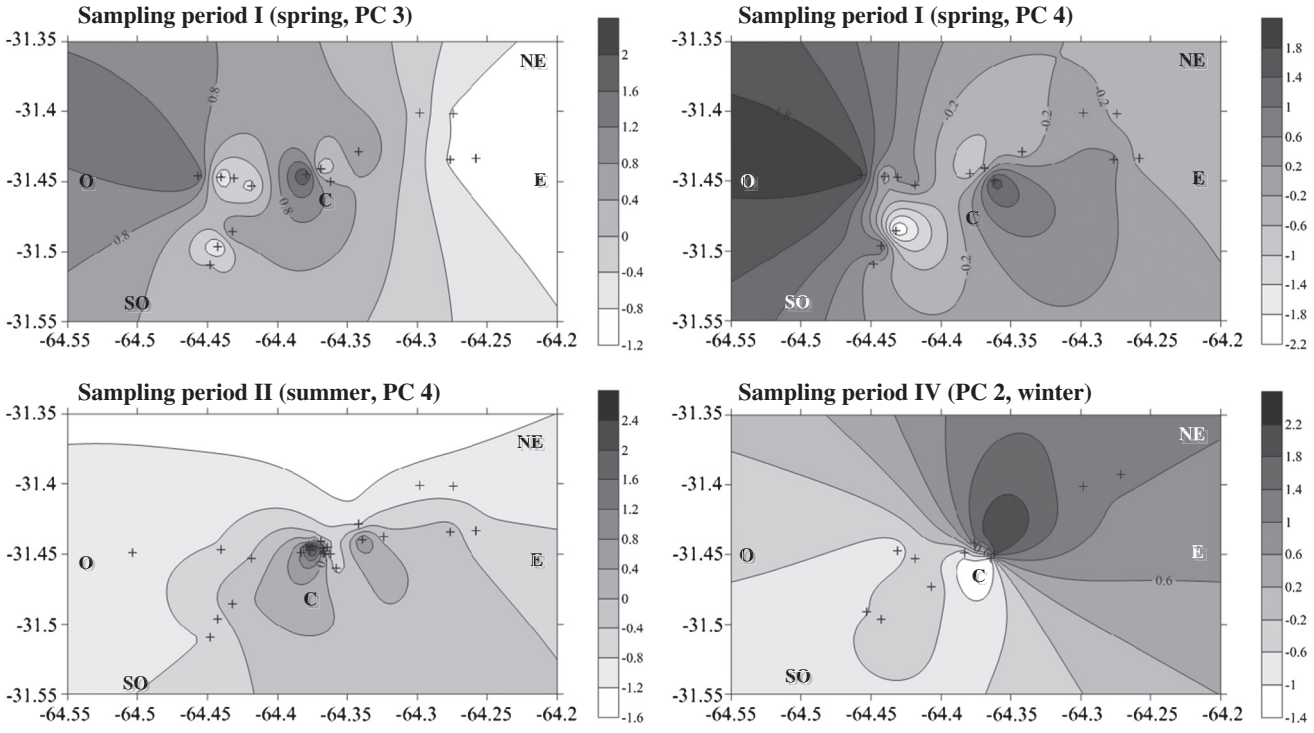

Fig. 4. Spatial distribution patterns of principal component values for the source of pollution identified: cement plant.

3.1.1. Low molecular weight PAHs (LMW PAHs)

In the sampling period II (summer), significant differences were observed in the concentration values of ACN between subareas, with the highest values being found at the subarea C followed by those at SW (Table S5). With respect to the concentration values of FLN, all the sampling periods showed significant differences, with subareas SW and W presenting the highest levels. With respect to PHE, at the C subarea (in autumn) there were two sites located in the vicinity of one of the stacks of the cement plant that showed markedly higher values of this compound compared to the rest of the sampling sites, which was probably due to the biomonitors being exposed to building downwash effects, as already hypothesized in Abril et al. [23]. Similar results were also found for ANT, where the maximum values were found at C in summer.

3.1.2. High molecular weight PAHs (HMW PAHs)

Significant differences were found for PYR only in spring, with maximum values occurring towards E, W and SW (Table S5). The highest concentration values of BaA were observed in the colder seasons (autumn and winter). Also, in terms of differences between subareas, significantly higher mean values of BaA were observed at C, which may indicate that the cement plant is a major source of emission of this compound. With regard to CHR and BbF, winter showed significant differences, with the maximum concentration values being found at C and W. Furthermore, the maximum concentration values of BaP, DBA and BghiP were found in the vicinity of the cement plant (C) and E, which may indicate that the cement plant and vehicular traffic were the two main sources of atmospheric emission of these pollutants in the study area.

3.2. E–B ratios

With the aim of identifying the highest accumulation levels of the PAH compounds in *T. capillaris*, Figs. S6 to S9 illustrate the enrichment factors (E–B ratios) by subarea for the four sampling periods. ANT and BaP were the most enriched compounds throughout the four seasons, implying that they were being continuously emitted in the study area. ANT was enriched in all the subareas during spring, autumn and winter, while in summer it was prominent in C; BaP was also enriched towards C and E. Other notable E–B ratios were FLT (enriched at C in spring and summer), BaA (enriched at C), FLN (enriched towards SW) and BkF and DBA (enriched towards E and C).

3.3. Principal Component Analysis and factor assignment

In Table 2 the results of a Principal Component Analysis (PCA) of the PAH concentrations to try to identify possible atmospheric pollution sources and the associations between the different sites are shown, with varimax rotation being applied to improve the differentiation between sources. Figs. 3 to 6 show the spatial mapping of the principal component value patterns for each source of pollution identified and characterized. It can be observed through the PCA analysis that between seasons there was a large variability in the PAHs associated with subareas (and sources), which may have been due to several factors, such as: weather conditions; behavior of sources (presence or absence of emissions in the case of waste dumping site burning or changes in fuel composition in the case of the cement plant); and location of sampling sites. However, some noticeable trends in PAH associations were detected and are described below.

(1) *Traffic emissions (NE and E): BkF, BbF, BaP, DBA and BghiP* (principal components illustrated in Fig. 3).

In a previous study using *T. capillaris* it was observed that 5 and 6 ring compounds such as BbF, BkF, BaP, DBA and BghiP showed higher concentration values at sites with high vehicular traffic

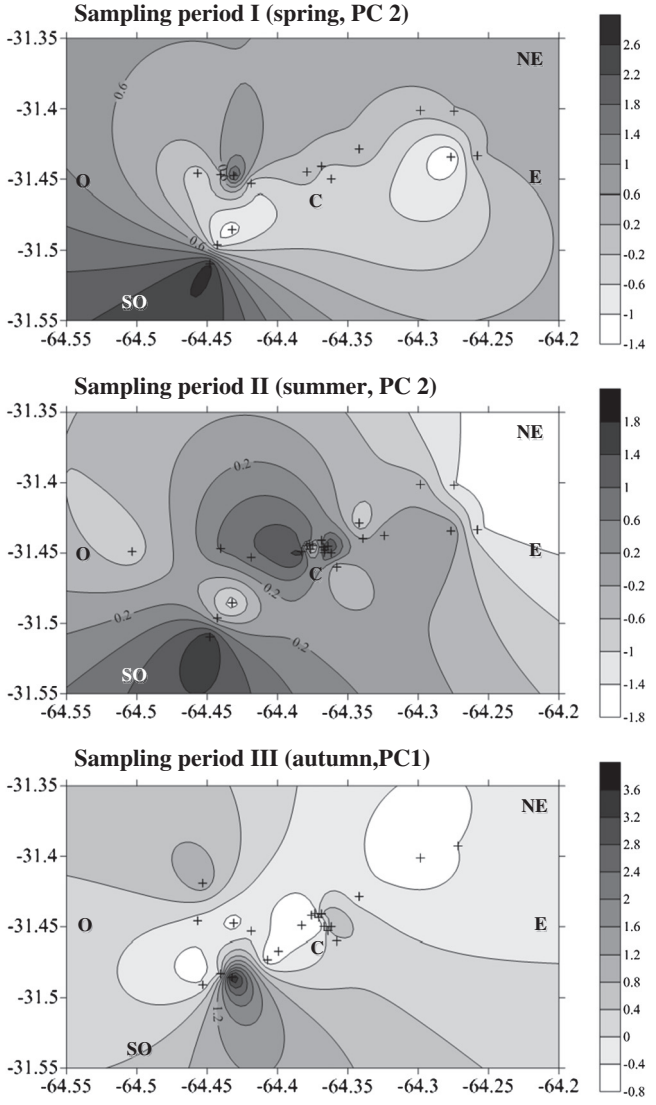

Fig. 5. Spatial distribution patterns of principal component values for the source of pollution identified: brick kiln emissions.

[11]. In addition, several other authors have agreed that high loads of BbF, BaP and BkF represent diesel emissions [34–36] whereas DBA and BghiP are indicators of gasoline vehicle emissions [37]. Biomonitors were transplanted to the E transect in the present study only in the first 2 sampling periods (spring and summer). Therefore despite the fact that these compounds were later related to the cement plant in autumn and winter, we mainly considered these marker elements to be mainly due to vehicular traffic emissions.

(2) *Cement plant (C): BaA, FLT, ACN, BaP* (principal components illustrated in Fig. 4).

FLT and BaA are related to combustion processes [38,39] and according to Yang et al. [40] ACN is mostly associated with emissions from cement plants. Wannaz et al. [14] conducted a study at different sites of the province of Córdoba using this biomonitoring species and passive air samplers, and found that BaA was a marker compound of the emissions of this cement plant. While other PAHs were also associated with the subarea C, these had seasonal variations and therefore could not be considered to be markers of this industry (PHE, CHR, PYR and DBA).

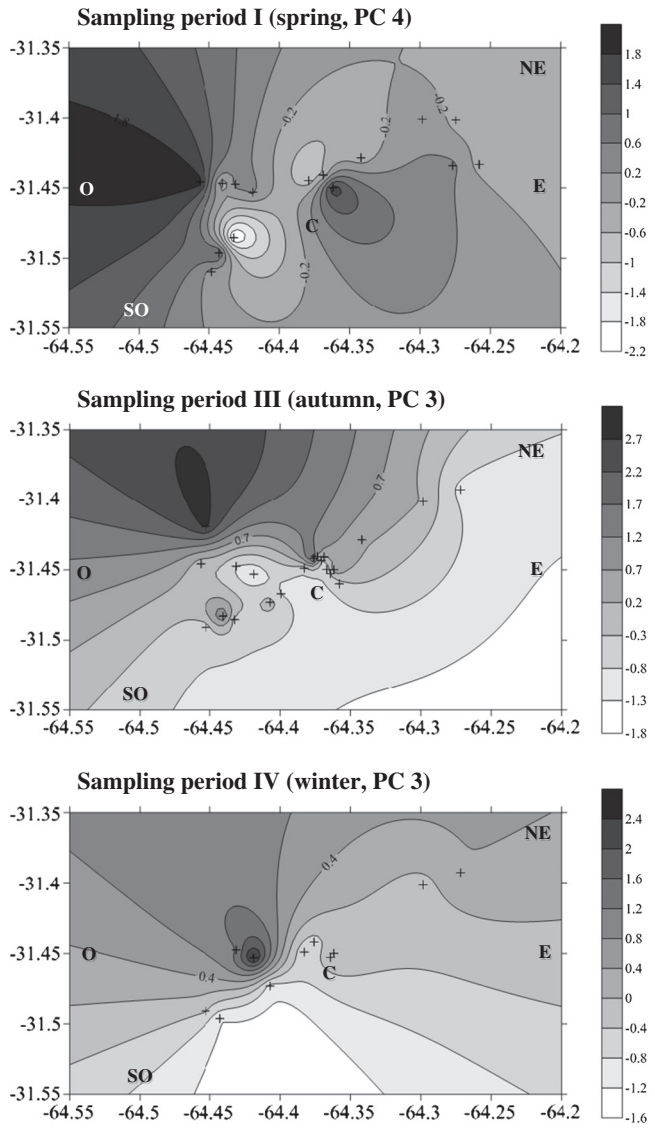

Fig. 6. Spatial distribution patterns of principal component values for the source of pollution identified: waste dumping site fires.

The variability of these compounds may have been due to several factors, such as the type of fuel, additives, manufacturing process, and atmospheric pollution control devices, with the most influential possibility being the fuel type. At this particular cement plant, the variability of the conditioned and used industrial wastes could also have had a decisive effect on the different compounds emitted and their temporal variabilities.

(3a) *Biomass burning (fires at the waste dumping site to the W): ANT, CHR, PYR and FTN* (principal components illustrated in Fig. 5).

(3b) *Biomass burning (brick kiln emissions to the SW): BbF, BkF, CHR, PYR, FTN and FLN* (principal components illustrated in Fig. 6).

The occurrence of FLN, CHR and PYR is an indicator of biomass burning [36,38,41], which in our study may be indicating the influence of emissions from brick kilns to SW and from fires to W, and with respect to ANT, previous studies have associated this with wood combustion [42,43]. In summer there was no PAH associated with the W subarea in our investigation, coinciding with the fact that in this season no fires were reported at the waste dumping site by the local fire station.

Table 3
Physiological parameters measured in *T. capillaris* in this study (a) and from other investigations in other regions of the province of Cordoba (b). Mean values ± standard deviation or ranges of the data are presented.

Variables	Units	Malagueño[a]										Industry Córdoba[b,1]	Córdoba[b,2]			Cordoba[b,3] cement plant			
		Period I			Period II			Period III			Period IV									
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean ± S.D.	Year I Mean	Year II Mean	Year III Mean	August–October Mean ± S.D.	November–January Mean ± S.D.	February–April Mean ± S.D.	May–July Mean ± S.D.
Pheo-a	$mg \cdot g^{-1}$ DW	1.92	0.50	3.49	1.24	0.48	2.19	0.61	0.19	1.24	1.65	0.63	2.64		1.71	1.90	1.59				
Pheo-b	$mg \cdot g^{-1}$ DW	0.77	0.20	1.39	1.14	0.23	4.19	3.42	0.93	5.21	1.02	0.45	3.22		0.59	0.86	0.94				
HPCDs	$\mu mol \cdot g^{-1}$ DW	12.82	1.94	28.54	18.66	3.65	73.03	34.67	10.13	62.87	25.05	11.81	49.24		31.38	37.05	23.27	33.66 ± 10.22	35.93 ± 5.02	37.71 ± 7.29	36.21 ± 10.63
MDA	$nmol \cdot g^{-1}$ DW	112.0	71.0	229.0	104.8	25.15	191.6	86.35	44.59	165.4	83.18	52.21	130.8	89 ± 5	143.2	104.9	163.9	111 ± 30	161 ± 31	79 ± 27	312 ± 94
S	$mg \cdot g^{-1}$ DW	1.22	0.51	2.41	0.88	0.28	1.60	0.74	0.30	1.37	0.71	0.26	1.27	2.235 ± 0.107	1.81	1.53	1.23	1.744 ± 0.640	2.289 ± 0.269	1.366 ± 0.218	0.881 ± 0.269
FDI	–	2.10	0.47	4.47	3.60	0.87	11.08	4.42	1.85	9.65	3.78	1.83	8.25	1.390 ± 0.183				2.056 ± 0.665	2.049 ± 0.268	1.744 ± 0.196	7.435 ± 1.936
pH	–	7.10	5.95	8.40	6.29	5.68	7.81	6.60	4.74	9.65	6.88	5.63	10.71								
EC	$\mu S \cdot cm^{-1} \cdot mL^{-1} \cdot g^{-1}$ FW	0.41	0.08	1.27	0.34	0.07	2.59	0.38	0.06	1.32	1.65	0.35	9.33								

Sampling periods: I (spring); II (summer); III (autumn); IV (winter). S.D.: standard deviation.
[1] Bermudez and Pignata [50] and Bermudez et al. [27].
[2] Wannaz [46].
[3] Rodriguez et al. [51].

3.4. Physiological parameters measured in T. capillaris

The descriptive statistics of the physiological parameters analyzed in *T. capillaris* corresponding to the four sampling periods are presented in Table S7. A comparison with other studies undertaken in other regions of the province of Córdoba is presented in Table 3, with the results of the analysis of variance (ANOVA) using the factors "subareas" (C, SW, NE, E and W) and "sampling periods" (I, II, III and IV) being shown in Table 4. Chlorophyll in lichens and epiphytic plants is very sensitive to changes in environmental factors including air pollution, and Molisch [44] reported pheophytin to be elevated in gas damaged leaves [45]. While the Pheo-a values in this study were similar to those presented by Wannaz [46] for a study undertaken in a extensive region of the province of Córdoba, our Pheo-b levels were visibly higher, and within our study area, these levels were significantly greater at subareas related to biomass burning (SW and W). The increase in MDA and HPCD content is related to lipid peroxidation of cell membranes [47,48], which is associated with an increase in the permeability of membranes (produced by air pollutants), and entails a loss of water and nutrients from the leaves, producing an early senescence in plants [49]. The MDA concentrations in the present study were lower than those reported by Wannaz [46], as well as those found by Bermudez and Pignata [50] in *T. capillaris* exposed in an industrial area of the province of Córdoba. However, our MDA levels were similar to those published by Rodriguez et al. [51] in *T. capillaris* transplanted to a site in the town of Malagueño (Table 3). With respect to the HPCD values quantified in our study, these were similar to those found in previous studies using the same species [46,51]. The maximum values of MDA were found in the subareas C and W, with HPCDs presenting significantly higher values for the periods III (autumn) and IV (winter) in

Table 4
Analysis of variance (ANOVA) using the factors "sampling areas" and "sampling periods" for physiological parameters measured in *T. capillaris* in the town of Malagueño in the province of Córdoba.

	Period	C			SW			NE			E			W			ANOVA	Control		
		Mean ± S.E.			Mean ± S.E.			Mean ± S.E.			Mean ± S.E.			Mean ± S.E.			p - value[a]	Mean ± S.E.		
Pheo-a	I	1.83 ± 0.10		A	2.02 ± 0.19		A	2.34 ± 0.26		A	2.16 ± 0.18		A	1.75 ± 0.17		AB	0.1851	0.42 ± 0.37		B
	II	1.24 ± 0.04		C	1.16 ± 0.09		B	1.13 ± 0.11		C	1.33 ± 0.08		B	1.27 ± 0.11		B	0.528	1.56 ± 0.13		A
	II	0.58 ± 0.02		D	0.53 ± 0.04		C	0.53 ± 0.05		D	n/d			0.63 ± 0.04		C	0.1814	0.69 ± 0.06		B
	IV	1.57 ± 0.05	c	B	1.92 ± 0.09	a	A	1.60 ± 0.13	bc	B	n/d			1.89 ± 0.10	ab	A	**	1.69 ± 0.16		A
p-Value[b]		***			***			***			***			***				***		
Pheo-b	I	0.74 ± 0.04		C	0.81 ± 0.07		B	0.94 ± 0.10		B	0.85 ± 0.07		B	0.70 ± 0.06		D	0.2128	0.18 ± 0.14		D
	II	1.08 ± 0.06	b	B	0.98 ± 0.12	b	B	0.94 ± 0.14	b	B	1.22 ± 0.11	b	A	2.04 ± 0.14	a	B	***	1.22 ± 0.17		B
	II	3.41 ± 0.10	b	A	4.00 ± 0.19	a	A	3.19 ± 0.25	b	A	n/d			3.69 ± 0.19	ab	A	*	3.86 ± 0.31		A
	IV	0.85 ± 0.08	b	C	1.22 ± 0.13	a	B	0.71 ± 0.20	b	B	n/d			1.29 ± 0.15	a	C	**	0.81 ± 0.24		C
p-Value[b]		***			***			***			***			***				***		
HPCDs	I	14.81 ± 0.85	a	D	11.81 ± 1.64	ab	C	13.06 ± 2.32	ab	B	11.66 ± 1.64	ab		8.49 ± 1.47	b	C	**	6.62 ± 3.28		D
	II	19.36 ± 1.44		C	17.74 ± 3.06		C	18.69 ± 3.53		B	15.54 ± 2.73			27.99 ± 3.53		B	0.1071	11.33 ± 4.32		C
	III	34.86 ± 0.93	b	A	34.85 ± 1.71	b	A	34.95 ± 2.21	b	A	n/d			42.16 ± 1.71	a	A	**	27.84 ± 2.70		A
	IV	23.42 ± 0.89	b	B	28.97 ± 1.42	a	B	17.33 ± 2.17	a	B	n/d			31.60 ± 1.68	a	B	***	19.38 ± 2.66		B
p-Value[b]		***			***			***			0.1757			***				***		
MDA	I	118.5 ± 4.21	ab	A	99.44 ± 8.15	bc	B	97.40 ± 11.52	bc	A	88.40 ± 8.15	c	B	126.8 ± 7.29	a	A	**	37.24 ± 16.29		C
	II	111.2 ± 3.55		A	120.7 ± 7.52		A	99.00 ± 8.68		A	102.4 ± 6.73		A	107.4 ± 8.68		B	0.3067	63.61 ± 10.64		A
	III	90.12 ± 2.55	a	B	70.10 ± 4.71	b	C	83.03 ± 6.08	ab	B	n/d			91.81 ± 4.71	a	B	**	51.95 ± 7.45		B
	IV	87.70 ± 1.80	a	B	78.26 ± 2.89	b	C	83.28 ± 4.41	ab	B	n/d			73.74 ± 3.42	b	C	**	64.43 ± 5.40		A
p-Value[b]		***			***			**			**			***				**		
S	I	1.28 ± 0.05	b	A	1.19 ± 0.10	b	A	1.10 ± 0.14	bc	A	1.60 ± 0.10	a	A	0.81 ± 0.09	c	A	***	1.05 ± 0.19		A
	II	0.91 ± 0.03	b	B	0.67 ± 0.07	c	B	0.80 ± 0.08	bc	B	1.22 ± 0.06	a	B	0.83 ± 0.08	bc	A	***	0.68 ± 0.09		B
	III	0.85 ± 0.02	a	BC	0.70 ± 0.03	b	B	0.72 ± 0.05	b	B	n/d			0.61 ± 0.03	b	B	***	0.60 ± 0.06		B
	IV	0.75 ± 0.03		C	0.72 ± 0.05		B	0.67 ± 0.08		B	n/d			0.76 ± 0.06		AB	0.7783	0.45 ± 0.10		C
p-Value[b]		***			***			*			***			*				***		
FDI	I	2.34 ± 0.11	a	C	1.82 ± 0.22	bc	B	1.27 ± 0.31	c	B	2.23 ± 0.22	ab	B	1.79 ± 0.19	bc	C	**	-		
	II	3.79 ± 0.25		B	3.84 ± 0.53		A	3.17 ± 0.61		A	3.84 ± 0.47		A	3.51 ± 0.61		AB	0.8975	1.55 ± 0.74		B
	III	4.70 ± 0.19	a	A	3.80 ± 0.38	b	A	4.24 ± 0.44	ab	A	n/d			4.13 ± 0.34	ab	A	*	1.97 ± 0.54		A
	IV	4.24 ± 0.17	a	B	3.21 ± 0.27	b	A	3.29 ± 0.41	b	A	n/d			3.26 ± 0.31	b	B	**	2.14 ± 0.50		A
p-Value[b]		***			**			**			***			***				**		
pH	I	7.49 ± 0.06	a	AB	6.76 ± 0.11	b	A	6.44 ± 0.15	b	A	6.73 ± 0.11	b	A	6.76 ± 0.10	b	A	***	6.02 ± 0.22		B
	II	6.54 ± 0.06	a	C	6.06 ± 0.15	b	BC	6.03 ± 0.15	b	B	6.05 ± 0.12	b	B	6.08 ± 0.15	b	B	***	6.07 ± 0.18		B
	III	7.19 ± 0.14	a	B	5.91 ± 0.25	b	C	5.48 ± 0.32	b	C	n/d			6.12 ± 0.28	b	B	***	6.54 ± 0.40		A
	IV	7.66 ± 0.12	a	A	6.28 ± 0.17	b	B	5.87 ± 0.32	b	B	n/d			6.19 ± 0.20	b	B	***	5.84 ± 0.32		C
p-Value[b]		***			***			***			***			**				***		
EC	I	0.49 ± 0.03	a	B	0.28 ± 0.06	b	B	0.36 ± 0.09	ab	B	0.41 ± 0.06	ab	A	0.28 ± 0.06	b	B	**	0.11 ± 0.13		B
	II	0.41 ± 0.05		B	0.42 ± 0.11		B	0.38 ± 0.11		B	0.24 ± 0.09		B	0.20 ± 0.11		B	0.2638	0.18 ± 0.13		B
	III	0.45 ± 0.03	a	B	0.28 ± 0.06	b	B	0.19 ± 0.08	b	B	n/d			0.33 ± 0.07	ab	B	*	0.16 ± 0.10		B
	IV	2.43 ± 0.21	a	A	0.83 ± 0.31	b	A	0.99 ± 0.59	b	A	n/d			0.73 ± 0.38	b	A	***	0.64 ± 0.59		A
p-Value[b]		***			***			***			**			***				***		

Sampling periods: I (spring); II (summer); III (autumn); IV (winter).
Lowercase letters are used to compare sampling areas. Capital letters are used to compare sampling periods.
n/d: no data.
[a] Values on each horizontal line followed by the same lowercase letter do not differ significantly (p = 0.05).
[b] Values on each column followed by the same capital letter do not differ significantly (p = 0.05).
* Significant at 0.05 probability level.
** Significant at 0.01 probability level.
*** Significant at 0.001 probability level.

W than those found in the other subareas. The maximum values of EC were found in C in periods I (spring), III (autumn) and IV (winter), which may have been due to the impact of emissions from the cement plant, and/or to the heavy loads of particulate matter emitted by this industry. In addition, the maximum values of pH in *T. capillaris* leaves were found in the vicinity of the cement plant during the four sampling periods, indicating that the effects from the alkaline cement dust had markedly local effects. As for the sites located in the subarea with the highest intensity of vehicular traffic (E, one of the entrances to the City of Córdoba), these showed the maximum values of S, which were similar to those reported by Wannaz [46], but lower than those obtained by Rodriguez et al. [51] at a site in the vicinity of this cement plant using *T. capillaris*. The FDI values obtained in the present study were higher than those found by Wannaz [46] in *T. capillaris* for a large region of the province of Córdoba and that those reported by Bermudez et al. [27] in *T. capillaris* exposed in urban and industrial areas of the province of Córdoba. Moreover, our FDI results were also higher than those obtained by Rodríguez et al. [51]. In another study, Bermudez [52] conducted a biomonitoring study using *Usnea amblyoclada* and found that samples transplanted into Malagueño (Yocsina) revealed values of a Pollution Index significantly higher than those present in other areas of the province of Córdoba, coinciding with the results presented here, where the FDI values were significantly higher in the vicinity of the cement plant.

4. Conclusions

The following sources of polycyclic aromatic hydrocarbons (PAHs) were characterized in the study area through their quantification in biomonitors and by measuring the parameters that could indicate physiological damage:

- Cement plant (C): ACN, BaA, FLT and BaP were the PAHs identified and FDI, MDA and pH the indicators of foliar damage resulting from the exposure to these air pollutants.
- Waste dumping site fires (W): ANT, CHR, PYR and FTN, and brick kiln emissions (SW); BbF, BkF, CHR, PYR, FTN and FLN were the PAH markers of biomass burning. In summer, in the W subarea, no PAHs were found to be associated, coinciding with the fact that no fire episodes were recorded. For both these sources involving biomass burning, Pheo-b, and HPCDs were the parameters indicating foliar damage from exposure to these air pollutants. In addition, these sources involved biomass burning, which was reflected in the emissions of these compounds, as previously reported by Abril et al. [23] with the accumulation of same metals and trace elements in the present study in the subareas SW and W.
- Vehicular traffic (E and NE): BaP, BkF, BghiP and DBA were the main markers of vehicle emissions, with BaP, and to a lesser extent BkF indicating diesel emissions, whereas DBA and BghiP showed emissions from gasoline vehicles. S was the main physiological parameter that indicated exposure to this source of emission of air pollutants.

For the PAHs associated with the identified emission sources in the town of Malagueño, it should be emphasized that from the most enriched PAHs, BaP is Category 1 for human carcinogenicity, as established by the International Agency for Research on Cancer (IARC, part of the World Health Organization).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.microc.2014.04.008.

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